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Transport characteristics of the thin limbs of Henle

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The loop of Henle is, in part, composed of two functionally distinct thin limbs, the thin descending (DLH) and thin ascending (tALH) limb of Henle. Each of these segments has a unique and a critical role in the overall operation of the countercurrent multiplication system. The vast majority of the published information concerning the quantitative aspects of the mechanism of electrolyte and water transport across these segments has been obtained by in vitro microperfusion techniques. In view of their different transport properties, it is convenient to consider these segments separately.

Thin descending limb of Henle

The characteristics of the rabbit in vitro DLH have been examined by morphological, structural, electrophysiological, and conventional in vitro microperfusion techniques.

During dissection the rabbit DLH can be identified easily by its attachment to the pars recta. It is much easier to hook up these segments with electrically tight seals (Sylgard) if a small segment of the pars recta is left attached and sucked into the holding pipette. Unfortunately, it is exceedingly difficult to dissect segments of DLH of other species than the rabbit because of the fragile nature of the pars recta—DLH transition junction.

The DLH appears as a tubule lined with flat epithelium with clear thin cytoplasm with only semirigid nuclei protruding into the lumen, Figure 1 [1]. Its outside dimensions are critically dependent on the applied perfusion pressure. At low perfusion pressures of 10 cm H_2O, the outside tubule diameter is approximately 75% of its maximum diameter achieved at 50 cm H_2O [2]. However, in contrast to the proximal tubule, the optical inside diameter is a close approximation of the true diameter in view of the close agreement with optically determined and calculated (obtained by cable analyses using electrophysiological measurement [3]) diameters. The freeze-fracture electron microscopy techniques of isolated DLH of rabbit have revealed morphologic features of the tight junction similar to that of electrically high resistance epithelia [3].

From a functional viewpoint both active and passive transport characteristics have been evaluated using in vitro microperfusion techniques. Evidence against active transport has come from a group of studies which noted that: *(1)* The transepithelial PD was near zero when perfusate and bath had similar ionic constituents; *(2)* net fluid transport was not statistically different from zero with similar solution on both sides of the epithelium; and *(3)* the collected fluid sodium concentration remained unchanged when perfused with isosmotic ultrafiltrate of some rabbit serum which was used in the bath [1]. Thus these studies suggested that DLH does not participate in active addition of solute into the papillary interstitium.

The passive permeability characteristics of the DLH are quite interesting. However, before describing the results of these studies, it is pertinent to point out that the DLH epithelium is quite fragile during dissection. It is now conventional to use Trypan Blue (0.25%) in the perfusate. This will allow for rapid detection of a macroscopic epithelial tear. The use of Trypan Blue has the added advantage that not only does it identify rapidly macroscopic hydraulic holes but it also stains nonviable epithelial cells. Descending limbs with cells which take up the Trypan Blue stain should be discarded. Unless extreme care is exercised it is not at all unusual to hook up a DLH only to note a hydraulic leak of colored perfusate and an associated leak of volume marker to the bath. It indeed is unacceptable to have any leak of volume marker when passive permeability characteristics are examined. A macroscopic hole in the epithelium not only would allow for transepithelial leak of a volume marker but also would not allow substances to exert their theoretical osmotic force. Thus it is imperative to use stringent acceptability criteria for inclusion of tubules into a study protocol.

We initially reported that the osmotic water permeability of the DLH was much greater than similarly determined water permeability of the proximal convoluted tubule [1]. In these studies net water movement was induced by the addition to the bath of either 100 mOsm of raffinose or sodium chloride. In each of the tubules examined there was no detectable volume marker leak. The determined Lp was 1.7 (with raffinose) and 1.6 (with sodium chloride) $\times$ 10^{-4} ml cm^{-2} sec^{-1} atm $^{-1}$. These studies were extended subsequently by using similar urea gradients to induce net water movement [4]. The results of these studies point out that water is significantly more permeable across DLH than sodium chloride, raffinose, and urea by showing that the ratio of rise in volume marker concentration was similar to the rise in total osmolality ratio. Similar results have been obtained by Abramow and Orci [3].

Stoner and Roch-Ramel [5] have reported that the water permeability of the DLH is pressure sensitive. At low perfusion pressures they argue that the DLH is relatively impermeable to water and that at higher pressures the Lp increases. They utilized the perfusion reservoir height as an index of intralu-

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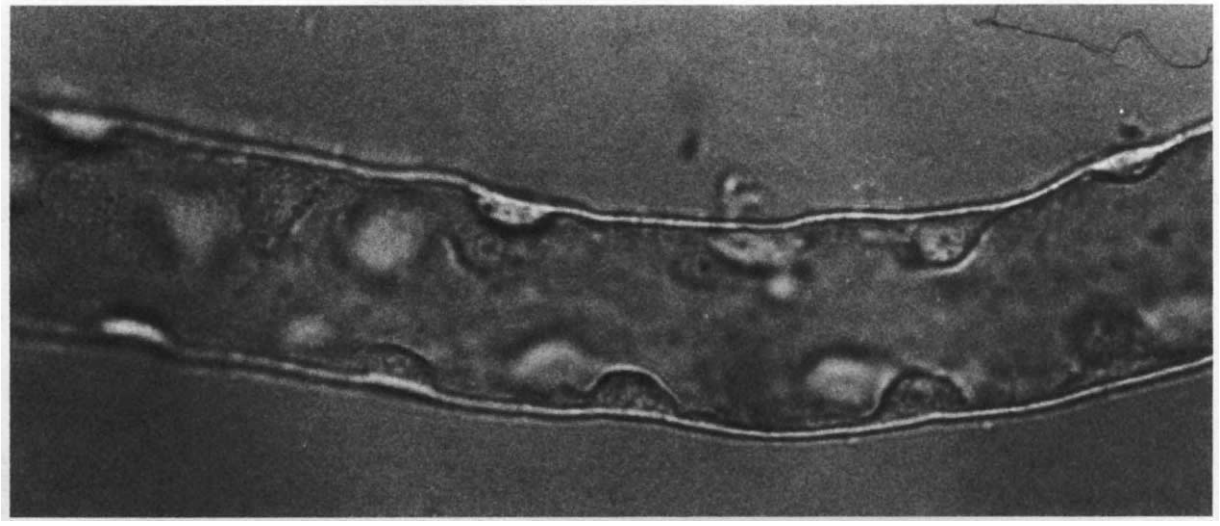

Fig. 1. *The appearance of an in vitro microperfused rabbit thin descending limb of Henle* [1]. (Magnification, ×400)

Table 1. Determinants of transport across thin limb of Henle as determined by in vitro microperfusion techniques

	Thin descending limb	References	Thin ascending limb	References
Active transport[a]				
Transepithelial PD	0	1	0	8, 10, 16
Net solute movement	0	1	0	8, 10, 16
Na flux	Bidirectionally equal	1	Bidirectionally equal	16
Passive transport				
Osmotic water permeability, Lp $\times 10^{-4}$ *ml* cm^{-2} sec^{-1} atm^{-1}	1.6 to 1.7	1	0	8, 10
Passive permeability coefficients Px $\times 10^{-5}$ *cm/sec*				
P_{Na}	0.16 to 1.9	1, 3, 6	22 to 80	8, 10, 16
P_{Cl}	ND		93 to 196	8, 10, 16
P_{urea}	1.0 to 1.5	4, 5	7 to 22	8, 10
P_K	2.5	6	Equal to P_{Na}	10[b]
P_{Ca}	0.8	7	1.4	7
P_{PO_4}	0.5	7	0.6	7
Reflection coefficient, ΣX				
ΣNaCl	0.95	1	NA	
ΣUrea	0.96	1	NA	
Transepithelial electrical resistance Ω cm^2	700	3	ND	

[a] Active transport was determined with identical luminal and bath solutions, ND, not determined; NA, not applicable since osmotic water permeability is zero.

[b] Value was electrophysiologically determined.

minal pressure. Unfortunately, they did not measure directly the luminal pressure and therefore their suggestions are indiret at best. The reservoir height does not reflect directly the intraluminal pressure. An additional problem with their studies is a significant loss of volume marker during control conditions. They report a control Jv = −0.29 nl/mm min which is equal to −0.54 nl/min for their individual tubules (corrected for average length of 1.86 mm). With an average perfusion rate of 4.36 nl/min this reflects an unacceptably high volume marker leak. Further, if one examines the rise in osmolality in the perfusate when raffinose was used in the bath, it is to be noted that the osmolality rise (84.1 mOsm/kg or 28%) was higher than predicted from net fluid movement. Thus, there was entry of raffinose. These results are not consistent with other in vitro studies and suggest that their DLH epithelium was somehow damaged during the dissection process.

The measured electrolyte and urea permeabilities of the DLH have been low in contrast to water permeability. These values can be calculated from the disappearance of the test radioisotope from the perfusate under conditions of zero transepithelial fluid flow [1, 4–7], or in net sense, from measurement of transepithelial resistance [3]. However, the determined perme-

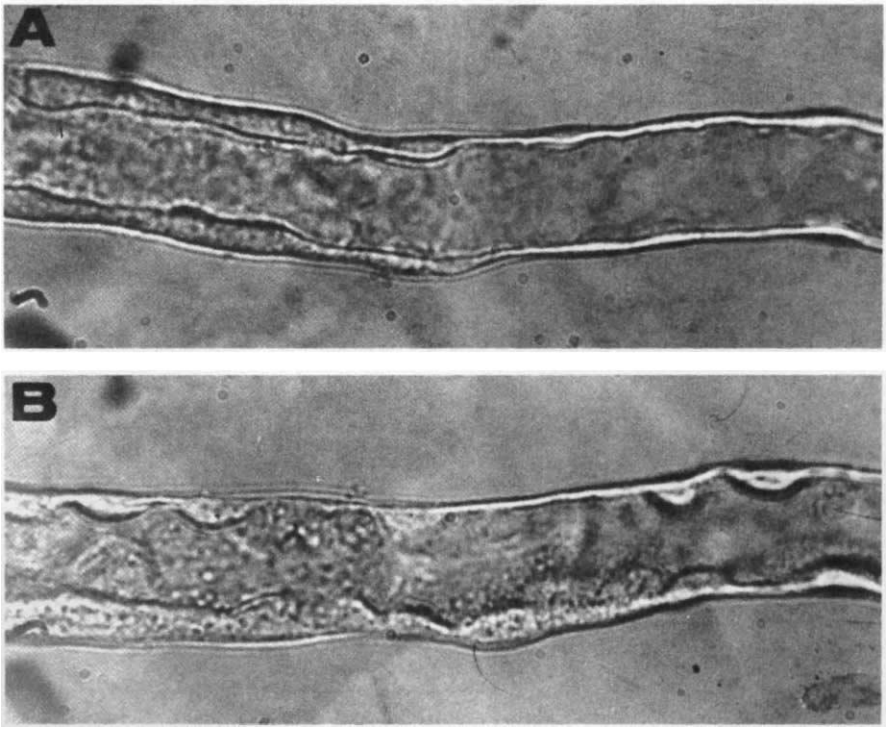

Fig. 2. *Contrast of the abrupt transition of the medullary thick ascending limb of Henle to that of the thin ascending limb of Henle epithelium* **(A)** *to that of a more gradual transition between the pars recta and the thin descending limb of Henle* **(B)** [8].

ability coefficients while low do not indicate complete impermeability of the DLH to solutes. These values are summarized in Table 1.

Of particular interest has been the permeability of the DLH to potassium and urea. When Rocha and Kokko [6] measured the potassium and sodium isotopic permeability of the same tubules, they were able to show potassium permeability was statistically somewhat higher than sodium permeability. Abramow and Orci [3] approached this same question somewhat differently. They imposed an osmotic gradient across the DLH with sodium chloride or potassium chloride and noted that the rise in volume marker ratio to osmolality ratio was much higher with sodium chloride than with potassium chloride thus concluding that potassium permeability is higher than sodium permeability. This would, in part, explain potassium recycling as proposed by in vivo studies.

Urea permeability is also quite low [4, 5] but not zero (Table 1). In addition, the reflection coefficient for urea is close to one [4]. Thus these studies are consistent with the view that urea can exert a significant osmotic force and abstract water out of the DLH while the measured urea permeability would allow for some diffusive influx of urea. It has been suggested that urea permeability might increase with increases in ambient osmolalities [5]. However, the basis of this suggestion comes from studies where urea permeability was measured under conditions of net fluid movement as induced by the addition of urea gradient to the bath. However, since solute can move in association with convective flow of fluid, it is not accurate to measure passive permeability coefficients with significant bulk movement of water. It is noteworthy that the transepithelial conductance (an index of electrolyte permeability) does not change with large stepwise changes in ambient osmolalities [3].

In summary, the transport characteristics of the DLH suggest that its main physiological function is to osmotically equilibrate fluid which courses through it. Since a significant portion of the medullary hypertonicity is accounted for by urea and since the overwhelming majority of the luminal osmolality is due to sodium chloride, the process of osmotic equilibration generates a fluid which is isosmotic to its surroundings but having a higher luminal concentration of sodium chloride. The thin ascending limb of Henle thus is operating under conditions with a favorable outward gradient for sodium chloride efflux.

Thin ascending limb of Henle

The thin ascending limb of Henle (tALH) has a pivotal role in the overall operation of the countercurrent multiplication system. Since this segment also is inaccessible to conventional micropuncture techniques most of the quantitative determinants of transport have been obtained by in vitro microperfusion techniques. Thin ascending limbs of Henle from rabbit, hamster, and rat have been studied.

The dissection of tALH is exceedingly difficult. These segments are dissected "downward" from the junction of the inner and outer medulla using the thick ascending limb as a point of positive identification. The downward direction is not easy due to the tightly adherent extensive capillary network. Creation of mechanical holes in the epithelium during dissection is not at all unusual. On light microscopy the tALH is hard to distinguish from the DLH. The epithelial cells of tALH appear somewhat flatter and the transition from the thick ALH to tALH is more abrupt than the transition from pars recta to DLH, Figure 2 [8].

One of the most important questions concerning the tALH is whether or not it actively transports sodium chloride [9]. Evidence for active transport would be the demonstration of net

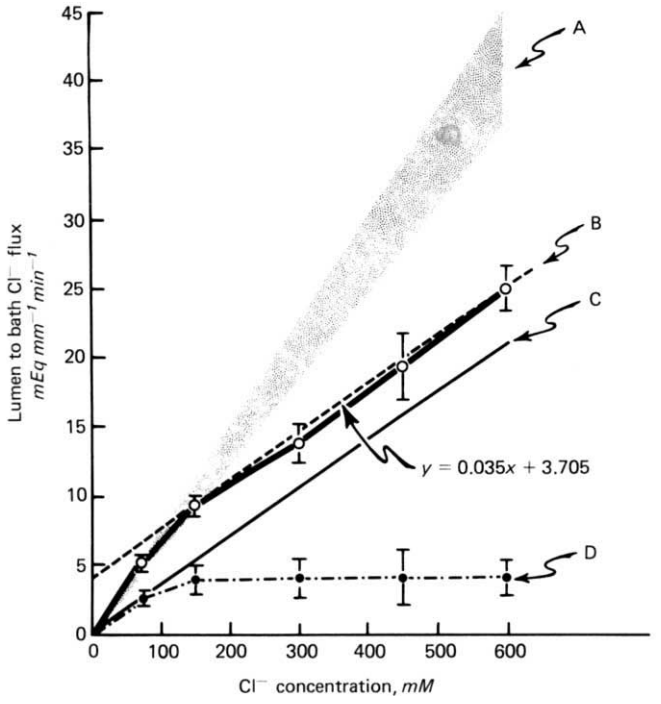

Fig. 3. *Lumen to bath chloride flux* (vertical axis) *as a function of ambient chloride concentration* (horizontal axis). **A** *Shaded area which indicates a range of chloride flux calculated from the mean and* SEM *obtained at the lowest chloride concentration based on the assumption that the chloride transport is in accord with simple massive diffusion.* **B** *Experimentally determined points* ± SEM. **C** *Parallel line to* **B** *which indicates the simple passive component of transport depicted by* **B. D** *is* **B** *minus* **C** *which reflects the component of chloride transport which follows saturation kinetics.* Calculated V_{max} = 3.71 mEq mm^{-1} min^{-1} [16]; $P_{Cl} = 92.9 \times 10^{-5}$ cm sec^{-1}.

solute movement in absence of external driving forces. However, when segments of tALH from rabbit [8], rat [10], or hamster [10] have been perfused in vitro with solutions identical to the bath, none of the data is suggestive of active transport. The transepithelial potential difference (PD) was zero, gravimetrically determined net solute movement was zero, and bidirectional fluxes of sodium and chloride were equal. Since it has been shown that vasopressin stimulates secondary active chloride transport in the thick ALH [11–13] and since vasopressin be published and [14]) it was logical to examine whether or not to examine whether or not the lack of ADH in the ambient media prevented the demonstration of active sodium chloride transport. Thus, Imai and Kusano [15] showed that while 1 mU/ml AVP increased the adenylate cyclase activity of tALH by 2.2-fold, there was no statistical change in either the transepithelial PD or ^{36}Cl efflux. Thus all of the in vitro studies have failed to support the existence of active sodium chloride transport out of the tALH.

The passive transport mechanism out of the tALH are quite unique. First this segment, in contrast to the DLH, is impermeable to osmotic flow of water. When segments of rat, rabbit, or hamster tALH were perfused in bathing solutions made hyperosmotic by the addition of 300 mOsm/kg raffinose, the net water movement was immeasureably small and the calculated Lp was not different from zero [8, 10].

The studies of passive sodium chloride and urea transport mechanisms out of the tALH have also yielded quite interesting findings. In an overview sense the tALH is moderately permeable to urea while being highly permeable to sodium and chloride [8]. In each of these cases the bidirectional fluxes of the respective isotopes has been equivalent when determined in the same tubule having solutions of similar composition on both sides. Furthermore, the permeability coefficient for sodium did not change when the osmolality of the perfusate and bath were raised simultaneously [15]. These results show that sodium (and probably urea) are transported across the tALH by simple diffusive mechanisms. However, the tALH is significantly less permeable to urea than to sodium (Table 1) and, therefore, for an equivalent driving force more sodium than urea would be transported.

Transport of chloride is much more complicated than the transport of sodium. The initial suspicion that chloride was transported passively by mechanisms other than purely passive diffusive mechanisms came from the observation that the unidirectional ^{36}Cl fluxes were several orders of magnitude higher (117×10^{-5} cm/sec) than chloride fluxes studied by similar techniques in other nephron segments [8]. Indeed this value is close to or higher than that of chloride-free diffusional mobility.

Studies, therefore, were designed to examine whether chloride transport occurs, in part, by some membrane interactive component such as single file diffusion or a carrier-mediated process (exchange diffusion or facilitated transport). Theoretically chloride transport could also be enhanced in association with solvent drag, but this mechanism is not applicable in a water impermeable segment.

If single file diffusion were to exist, the permeability coefficient for ^{36}Cl would increase as luminal and bath concentrations of chloride were increased. However, as luminal and bath chloride was increased successively in step-wise fashion from 75.5 to 598.6 mM/liter, the observed chloride permeability coefficient actually fell from 187.9 to 103.6×10^{-5} cm/sec [16]. This, therefore, is strong evidence against single file diffusion.

If exchange diffusion were responsible for high unidirectional chloride fluxes, then chloride flux should decrease with removal of bath chloride while maintaining the luminal chloride concentration unchanged. When this series of studies was conducted by replacing bath sodium chloride by mannitol the ^{36}Cl flux did not decrease which ruled out the significant component of exchange diffusion [16].

To evaluate for a facilitated transport process, we examined lumen to bath chloride flux as a function of bath and luminal chloride concentration (Fig. 3). It is quite clear from the experimentally determined points (line **B**) that the lumen to bath flux appears curvilinear in nature and made up of two components: line **C** which represents simple passive diffusion and line **D** which represents a component of chloride flux which follows saturation kinetics [16]. Furthermore, added luminal bromide inhibited the unidirectional efflux of ^{36}Cl. Thus these studies would lend strong support to the view that chloride transport across tALH occurs by at least two passive mechanisms: *(1)* simple passive diffusion and *(2)* carrier-mediated facilitated transport process. This latter process is not "active" since it is

unable to lower the chloride concentration in the absence of chloride concentration gradients [8].

In summary, the tALH transport characteristics are ideally suited for the addition of sodium chloride without water into the papillary interstitium. This solute addition can occur by purely passive mechanisms. Sodium chloride diffuses down its concentration gradient while some urea recirculates into the tALH. The net effect is to leave the tALH less concentrated since sodium chloride efflux would be greater than urea influx. For the tALH to function in this capacity, it is critical for it to receive fluid from the DLH which contains a sodium chloride concentration higher than in adjacent papillary interstitium.

Physiological significance

In conclusion, the above reviewed transport processes of the descending and ascending thin limbs of Henle clearly show that the epithelial characteristics of these two segments are entirely different. It is these experimental values which lead to the formulation of the countercurrent multiplication system in which both limbs act as purely passive equilibrating segments. Thus, the necessity of postulating active sodium chloride transport out of the tALH was removed as the primary energy source responsible for operation of the countercurrent multiplication system [17]. While additional factors may contribute to the formation of concentrated urine as initially postulated by the passive equilibration model of countercurrent multiplication system, the existing experimental finding and theoretical [17–19] evaluations are consistent with the general architectural features of this model as originally formulated.

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